EXPERIMENTS ON LIGIA IN BERMUDA

V. FURTHER EFFECTS OF SALTS AND OF HEAVY SEA WATER

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Four previous papers of this series (Barnes, 1932, 1934, 1935, 1936) have described aspects of the behavior, salt requirements and thermal range of *Ligia baudiniana*, an interesting isopod which is invading the land through the intertidal zone. The present report deals with further experiments on the capillary mechanism conveying sea water to the gills, reactions to filter paper saturated with diluted sea water, the protective action of Ca in hypotonic sea water and the combined effect of high temperature and heavy water.

HABITS

It was reported previously that the release of young from the brood pouch was observed only in specimens kept submerged in sea water, but during the past summer this phenomenon occurred in a few cases in females in air over filter paper moistened with sea water. On the other hand, the molting process has occurred only in air. No adult specimens have been taken in the sea, but in rare cases an isopod will enter sea water in a terrarium to feed.

Ligia is provided with a capillary mechanism for keeping the gills moist without entering the sea. The first paper of this series stated that the uropods and spines are lowered into the water or onto a water film and the sea water then rises by this capillary path to the gills. However, Mr. M. D. Burkenroad has drawn my attention to a more important capillary conduit by which the water rises between the sixth and seventh legs to the gills and is then propelled by the gills *down* the uropods and spines (Fig. 1, *A*). The sixth and seventh leg on one or both sides may be used. Frequently the animal slides one leg over the other alternately at the start. The drainage down the uropods was described in a former paper (Barnes, 1935) in experiments in which sea water was dropped on the animals. The flow was followed in the present experiments by the addition of stains, fine particles or bubbles to the sea water. If the uropods are lowered when the last pair of legs are not drawn together, the first movement of the water is up the capillary conduit of the uropods as originally described (which may be observed by the movement of particles in the sea water). When the isopod is totally immersed, the currents produced by the beating of the gills in the surrounding sea water (indicated by the movement of particles) follow the same direction as the capillary circuit involving the rise of water between the last pair of legs (Fig. 1, B).

The sixth or seventh leg was removed in some specimens to modify the method of obtaining water. In the absence of the seventh leg a specimen standing on moist filter paper usually takes up the position for the capillary circuit via the legs (Fig. 1, *C*). It feels about frantically with the sixth leg for the missing seventh leg to complete the capillary channel. No attempt is made to use the fifth leg. Similarly if the sixth leg is removed, the conduit is impossible. Some specimens "squat" on the water film and the water rises by capillarity on the whole undersurface of the body and gills (Fig. 1, *D*). Other specimens with the sixth leg missing lower the abdomen and uropods and thus

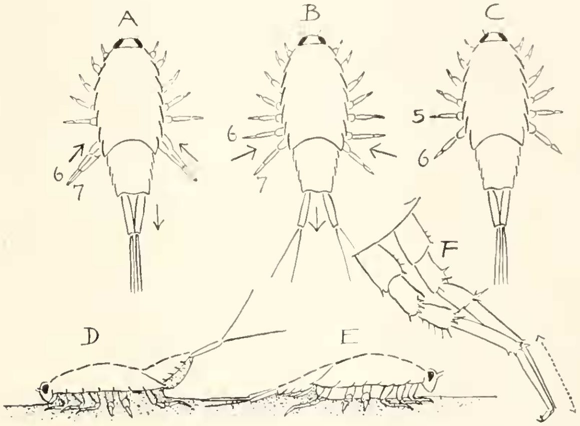

FIG. 1. Methods of moistening the gills in *Ligia*. (In these sketches the antennae and body segmentation are omitted.)

A. Specimen in air on filter paper saturated with distilled water. Water rises between the sixth and seventh leg by capillarity and is drained down between the uropods as indicated by arrows.

B. Specimen immersed in sea water. The arrows indicate the currents maintained by the beating of the gills.

C. Specimen with seventh leg removed standing on filter paper saturated with sea water. Sixth leg and uropods are in position for capillary circuit.

D. Side view of specimen with sixth leg removed standing on film of sea water (stippled).

E. Side view of specimen with sixth leg removed with abdomen lowered in film of sea water.

F. Sixth and seventh legs permanently fused along segment indicated by dotted line (found on left side of one specimen). These partially fused legs functioned normally as a capillary channel.

secure water by capillarity (Fig. 1, *E*). It is clear that there are several methods of wetting the gills. Indeed, many specimens are found which have lost the uropods and spines. These simply lower the abdomen on a damp substratum.

An interesting specimen was found in which the sixth and seventh leg were permanently fixed in the capillarity posture owing to the fusion of the last segments (Fig. 1, *F*). While walking, the double leg moved alternately with the seventh leg on the opposite side, but sometimes dragged. The joined legs functioned normally as a channel above the point of fusion.

REACTION TO FILTER PAPER SATURATED WITH DILUTED SEA WATER

Large filter papers (diameter 25″) were cut in two, one half saturated with distilled water, the other with diluted sea water and placed in a covered flat dish in a photographic darkroom having a light directly above the center of the dish. Four isopods (previously kept on seaweed moistened with sea water) were placed in the dish and their distribution on the two halves was observed at ten to fifteen-minute intervals over a period of one to two hours. The dish was rotated 90° after each reading to eliminate any unsuspected source of orientation. The animals showed a distinct tendency to collect on filter paper saturated with distilled water when the other half was moistened with sea water (Table I, and Barnes, 1935). It was found that this aversion was also shown to 75 per cent sea water, but not to dilutions of 50 per cent and below (Table I).

TABLE I

Reaction of Ligia to Filter Paper Saturated with Diluted Sea Water
(The animals were tested in groups of four)

Treatment of each half of paper	Total number of isopods found on each half	Ratio
Sea water *vs.* distilled water	69 : 123	1 : 1.78
75 per cent sea water *vs.* distilled water	27 : 73	1 : 2.74
50 per cent sea water *vs.* distilled water	93 : 96	1 : 1.03
25 per cent sea water *vs.* distilled water	109 : 73	1 : 4.9
10 per cent sea water *vs.* distilled water	27 : 21	1.28 : 1

The tendency to avoid sea water was most pronounced during the first observations in a given experiment and after an hour or more, when the paper containing distilled water was becoming dry, most specimens collected on the sea water side. Thus the ratio of specimens on the sea water side to those on the distilled water side was 1 : 3.3

for all the first readings and 2.3 : 1 for the last observation made after an average time interval of one and one half hours.

THE PROTECTIVE ACTION OF CALCIUM IN HYPOTONIC SEA WATER

Ligia survives for only about seven hours in 100 cc. of 25 per cent sea water, but as with many other forms, the addition of calcium protects the organism from hypotonic media, apparently by decreasing permeability. As in all the experiments with solutions, individual specimens were tested in 100 cc. of solution in finger bowls. As will be seen from Table II, there is a threshold for the Ca effect (at about 12 cc. $\frac{5}{8}$ M $CaCl_2$ added to a liter of 25 per cent sea water). It is also apparent that the addition of Ca above a critical quantity (20 cc. $\frac{5}{8}$ M $CaCl_2$ added to a liter of 25 per cent sea water) has little additional protective action. It is interesting to note (see Fig. 2) that the average survival in calcified 25 per cent sea water approaches the average longevity in 100 cc. of natural sea water.

TABLE II

Longevity of Ligia in 25 per cent Sea Water Containing Added $CaCl_2$

cc. $\frac{5}{8}$ M $CaCl_2$ added to 1 liter 25 per cent sea water	Average longevity	Maximum longevity	Coefficients of variation	Number of specimens
	hours	*hours*		
0	7 ± 0.33	15	4.7	33
2	6.5 ± 0.20	11	3.0	30
5	7.6 ± 0.37	17	4.8	58
12	24.6 ± 3.66	119	14.8	30
15	24.1 ± 3.70	144	15.3	20
20	51.5 ± 7.98	120	15.5	20
25	37.8 ± 5.09	168	13.5	30
50	38.5 ± 3.85	180	10.0	40

THE REVERSIBILITY OF THE CALCIUM EFFECT

Experiments were carried out to see if the protective action of calcium involves an irreversible chemical process. If so, preliminary treatment in solutions of high calcium content should lengthen the survival of isopods subsequently immersed in hypotonic sea water. However, it was found that preliminary immersion in calcified sea water from half an hour to over a day had little if any effect on the subsequent longevity of the treated specimens in 25 per cent sea water (see Table III). Likewise, preliminary treatment with sea water having a high content of sodium has no significant effect on subsequent action of hypotonic sea water. In all these tests many specimens died during the preliminary treatment so that those tested in the 25 per cent sea water represented a selected population capable of withstanding the submerged state. It must be remembered, however, that the survival of *Ligia* in any liquid medium is limited by unknown factors so that these selected specimens were already weakened by submersion. That these two factors balanced each other is indicated by the similarity of the average longevity in 25 per cent sea water of untreated and treated specimens. Thus the 152 isopods exposed to previous solutions survived 8.9 hours in 25 per cent sea water compared to the usual average of about seven hours in this medium for freshly collected individuals. In all the tests the specimens were washed by rapid immersion in distilled water after treatment in the calcium solutions, but this had no effect on the subsequent survival in 25 per cent sea water. Thus controls treated with ordinary sea water and

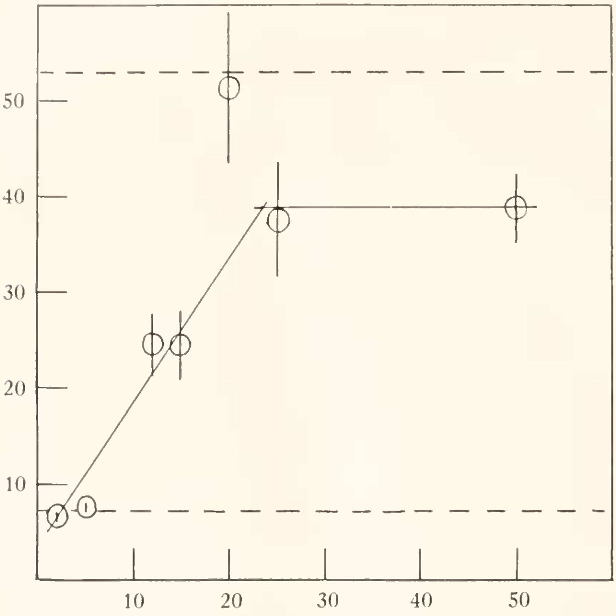

FIG. 2. Length of life of *Ligia* in 100 cc. of 25 per cent sea water with increasing amounts of $CaCl_2$.

Ordinates, average life in hours. Abscissæ, cc. of $\frac{5}{8}$ M $CaCl_2$ added to 1,000 cc. of 25 per cent sea water. The graph represents 228 tests (see Table II). Lower dotted line is the expected survival in 25 per cent sea water. Upper dotted line is the average survival in natural sea water. The vertical lines are proportional to the probable errors. The sea water average represents 265 specimens and the 25 per cent sea water average, 63 specimens. These are cumulative totals for five seasons' work. In all cases an individual specimen was tested in 100 cc. of solution.

then washed in distilled water lived the usual interval of about 7 hours in 25 per cent sea water.

Experiments in which the isopods were tested in distilled water after treatment with $CaCl_2$ give similar results (see Table IV). After exposure to $\frac{5}{8}$ M $CaCl_2$ the average survival was three hours in distilled water compared to 2.8 hours for untreated specimens.

THE COMBINED EFFECTS OF HIGH TEMPERATURE AND HEAVY WATER

It was previously shown that *Ligia* succumbs rapidly in a small volume of sea water at 38° C. The following experiments were performed to ascertain if sea water containing 99 per cent heavy water would protect the organism against heat death or else potentiate with high temperature by reducing the survival time. In each case 5 cc. of sea water were evaporated and the salts redissolved in ordinary distilled water or in 99 per cent heavy water. It was found that death ensued twice as rapidly in heavy sea water at 38° C. The survival times in 5-cc. samples were 23 minutes for H_2O sea water and eleven minutes for D_2O sea water (see Table V). Owing to the scarcity of heavy water, several specimens were tested in the same sample, but no appreciable difference was observed in the survival times of the first and last isopods treated.

TABLE III

Longevity of Ligia in 25 per cent Sea Water after Treatment in Calcified Sea Water

Initial solution	Average length of treatment	Subsequent average longevity in 25 per cent sea water	Maximum longevity	Coefficients of variation	Number of specimens
	hours	*hours*	*hours*		
Sea water	12.2	6.2±0.30	13.5	4.8	34
50 cc. $\frac{5}{8}$ M $CaCl_2$ plus 950 cc. sea water	31.5	5.5±0.32	6.5	5.8	13
100 cc. $\frac{5}{8}$ M $CaCl_2$ plus 900 cc. sea water	21.1	6.5±0.33	9	5.0	22
200 cc. $\frac{5}{8}$ M $CaCl_2$ plus 800 cc. sea water	17.6	9.2±0.38	17	4.1	49
500 cc. $\frac{5}{8}$ M $CaCl_2$ plus 500 cc. sea water	0.5	10.5±0.39	18	3.7	40
400 cc. $\frac{5}{8}$ M NaCl plus 600 cc. sea water	25.3	9.4±0.79	25	8.4	28

DISCUSSION

The tendency of the isopods to collect on filter paper treated with distilled water rather than on sea water paper is of interest in connection with the animals' aversion for the sea. The reaction appears to

TABLE IV

Longevity of Ligia in Distilled Water after Treatment with Calcium

Initial solution	Average length of treatment	Subsequent longevity in distilled water	Maximum longevity	Coefficients of variation	Number of specimens
	minutes	*hours*	*hours*		
None	0	2.8 ± 0.25	5.2	8.9	22
500 cc. $\frac{5}{8}$ M $CaCl_2$ plus 500 cc. sea water	33	4.5 ± 0.15	6	3.3	20
$\frac{5}{8}$ M $CaCl_2$	12	3 ± 0.20	4	6.7	12

be determined by the salt content of sea water since dilution of 50 per cent destroys the effect. It is possible that the salts on the paper stimulate the isopods to greater movement which would cause them to collect on the salt-free side. Gunn (1937) has described a hygrokinetic effect in *Porcellio scaber* whereby the greater activity of animals in dry air causes them to collect in moist locations, but this mechanism is probably not responsible for the reaction of *Ligia* described above. As was shown in a previous paper (Barnes, 1935), specimens will collect on dry filter paper when the other side of the dish contains filter paper moistened with distilled water. This refers to moist specimens. It is not known what part the flushing mechanism of the gills plays in these reactions. Bateman (1933) found that salts become slightly concentrated in the blood of specimens kept in air and it is possible that flushing the gills, especially with distilled water, enables the animal to get rid of salts concentrated by evaporation. On the other hand, specimens which have been immersed in distilled water also show the aversion for paper soaked in sea water (Barnes, 1935).

TABLE V

Longevity of Ligia in 5 cc. of Ordinary and Heavy Sea Water at High Temperature

Medium	Temperature	Average longevity	Maximum longevity	Coefficients of variation	Number of specimens
	° C.	*seconds*	*seconds*		
Sea water	38	1383 ± 45.1	2005	3.26	27
Sea water containing 99 per cent D_2O	38	660 ± 57.0	1140	8.63	11

The well-known action of Ca as a factor enabling organisms to withstand hypotonic solutions (for reference *cf.* Barnes, 1934) is strikingly illustrated by *Ligia*, which survives almost as long in 100 cc. of 25 per cent sea water of approximately 0.015 M $CaCl_2$ content as in 100 per cent of natural sea water. To the list of favorable artificial sea water solutions for *Ligia* described in previous papers may be added this new hypotonic mixture which is equivalent to sea water in which the Na, K and Mg content has been reduced to one fourth and the Ca content raised slightly. However, the *maximum* longevity in this medium is far short of the 297 hours observed in natural sea water. The very long survival of occasional specimens in sea water suggests that other factors besides salt effects are involved, such as the amount of food in the gut, the nature of the previous environment, the oxygen content, or the necessity for molting (which apparently does not occur in sea water). The high summer temperature of approximately 27° C. must also be considered.

The failure of preliminary immersion in sea water rich in calcium to protect the organism against subsequent exposure to hypotonic sea water indicates that Ca forms a loose, rapidly reversible combination with material in the plasma membrane. The existence of a threshold concentration and of a limited range in which the effect of Ca is proportional to the concentration suggests that a surface reaction is involved.

The rapid lethal action of 5 cc. of sea water at 38°, in which the ordinary water has been replaced by heavy water, was an unexpected result. The lower energy content of heavy water, which under certain conditions might be expected to protect an organism from high temperatures, was overbalanced by the well known toxic action of high concentrations of deuterium. Barbour (1937) has recently discussed the toxicology of heavy water. In larger volumes of sea water or even fresh water *Ligia* withstands high temperatures for a much longer period, probably on account of the greater quantity of oxygen present. The 5-cc. samples used in the tests were exposed to the air in a Petri dish so that the oxygen content of both heavy and ordinary sea water was probably the same.

SUMMARY

1. When presented with a "choice" between filter paper moistened with sea water or with distilled water, freshly caught specimens of *Ligia* tend to collect on the latter. Dilution of the sea water destroys this effect.

2. The survival of *Ligia* immersed in 25 per cent sea water with added calcium approaches the longevity in natural sea water.

3. Preliminary exposure of solutions of high calcium content does not protect *Ligia* from subsequent immersion in hypotonic sea water.

4. The lethal action of 5 cc. of sea water at 38° C. is enhanced by 99 per cent heavy water.

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